

linearized by *Not* I using T4 DNA ~~ligase~~ ligase resulting in a pPIC9K/P4H α expression vector. The direction of insert was identified.

¹⁶

Change(s) applied Please amend the paragraph beginning at page 38, line ~~15~~¹⁶, as follows:

/S.G.L/ to document,
4/13/2011
3) P4H β cDNA cloning and recombinant expression vector construction

According to the known chicken P4H β subunit gene sequence, the primers F: 5'GCG GCC GCA CAG CCC CTG GAG GAG 3' (SEQ ID No.24) and R: 5'GCG GCC GCG GTG ATG TAG ATC AGT C 3' (SEQ ID No.25) were designed and *Not* I restriction sites were introduced. RNA was extracted from sternum of 17-day-old chicken embryos followed by RT-PCR. 1.5 mM [Mg²⁺] was used in the PCR system.

Please amend the paragraph beginning at page 39, line 20, as follows:

PCR was performed using the plasmids pGEM-T/P4H α and pGEM-T/P4H β in Example 8 and 9, as templates. The primers' sequences were P4H α : F: 5'GC GGCCGC GAT ACT GCT ACG AAA G3' (SEQ ID No.26); R: 5'GC GGCCGC CTC CAA CTC TGA TAA C 3' (SEQ ID No.27); P4H β : F: 5'GC GGCCGC CAG CCC CTG GAG GAG -3 (SEQ ID No.28'); and R: 5' GC GGCCGC TTA ATC ATC ATC AGC 3' (SEQ ID No.29).

Please amend the paragraph beginning at page 40, line 14, as follows:

Since the P4H β expression unit was located upstream, PCR was used to examine whether the direction of P4H α and β was coincident. The upstream primer (5' GC GGCCGC CAG CCC CTG GAG GAG 3') (SEQ ID No.28) and downstream primer (5' GC GGCCGC CTC CAA CTC TGA TAA C 3') (SEQ ID No.27) for amplifying

Please amend the paragraph beginning at page 33, line 12, as follows:

- 1) The construction of the eukaryotic expression vector pPICZ α B/ CCOL2A1

The eukaryotic expression vector was constructed as follows: PCR was performed using the primers (sequence 25 5'GGT ACC TTG GTG GAA ACT TTG CGG 3'; SEQ ID No.18) and (sequence 26 5'GGT ACC GTT ACA AGA AGC AGA CTG 3'; SEQ ID No.19), and pGEM-T/CCOL2A1 as template. The PCR mixture contained 25 μ l of 2 \times GC Buffer I, 4 μ l of dNTP (2.5 mM), 0.5 μ l of 20 μ M primers.

Please amend the paragraph beginning at page 37, line 21, as follows:

According to the chicken P4H α gene sequence, primer a: 5'AGA TAC TGC TAC GAA AGA CCC CGA G 3' (SEQ ID No.20) and b: 5' CTC TCT TGG TTG TAG CCC TCA TCT G 3' (SEQ ID No.21) were designed. The PCR system contained 5 μ l of 10 \times PCR Buffer, 5 μ l of 25 mM MgCl₂, 4 μ l of 2.5 mM dNTP Mix, 0.5 μ l of 20 μ M primers, 1 μ l of cDNA ,0.5 μ l Taq, and water to a final volume of 50 μ l. PCR products were examined by 1% agarose electrophoresis.

Please amend the paragraph beginning at page 38, line 6, as follows:

The P4H α PCR product described above was ligated with pGEM-T, resulting in a pGEM-T/P4H α plasmid. A *Not* I restriction site was introduced by PCR. The primers a: 5'GCG GCC GCA GAT ACT GCT ACG AAA G 3' (SEQ ID No.22) and b: 5'GCG GCC GCC TCT CTT GGT TGT AGG 3' (SEQ ID No.23), and Pfu DNA polymerase were used for PCR. PCR products were given polyA tails then ligated with a pGEM-T vector and sequenced. The target fragments were recovered after the pGEM-T/P4H α was digested by *Not* I. The P4H α gene fragment was ligated with the pPIC9K vector